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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The long term goal of this project has been to define and explain mechanistically, interactions of the immune system with molecules related to "biological stress". During the tenure of this proposal, we demonstrated that norepinephrine (NE) can inhibit both T and B cell activation by mitogens, antigen, and interleukin 2. The use of a clonally derived T cell lymphoma, the S49 cell, and S49 cell mutants unable to synthesize cyclic AMP ($AdCy^-$) or utilize the cyclic AMP-dependent protein kinase (PKA^-) revealed that NE effects required the presence of a functioning cyclic AMP/protein kinase A system. Examination of the NE-mediated down regulation of the Thy-1 gene revealed that NE modulation occurs at mRNA transcription and requires the presence of a functional cyclic AMP protein kinase. In contrast, although NE also inhibited B cell activation, such inhibition was found to be less dependent on cyclic AMP, suggesting an alternate signal transduction pathway. Parallel studies, utilizing a MOPC-315 cell system for studying anti-tumor immunity showed that NE also (continued)

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lymphocyte activation, Thy-1 protein, cyclic nucleotides

Block 19 continued from front
down-regulated an anti-tumor response by immune cells. Unexpectedly, none of the NE responses described above were reversible by the β-adrenergic antagonist, propranolol, suggesting possible alteration of the normal properties of the β-adrenergic receptor during lymphocyte activation or involvement of a different receptor. During the course of these studies, our mouse colony was infected by murine hepatitis virus (MHV). In the presence of MHV, spleen T cells showed diminished mitogenic response and an ablated NE response whereas thymic cells and spleen B cells were unaffected. In total, these experiments define base lines for further studies in stress-related immune function in terms of both signal-transduction mechanisms and tumor immunity and suggest potential avenues for therapeutic modalities for stress-related pathology.

Final Progress Report - Interactions of Neuromodulators with Cells of the Immune System (N00014-87-0027) D.A. Chambers and R.L. Perlman

INTRODUCTION

The long term objectives of this research are to understand the molecular mechanisms through which "stress" affects the immune system. A variety of studies have led in recent years to the appreciation that molecules associated with the "stress" response, such as catecholamines, can have profound effects on immune function. Indeed, a new interdisciplinary area of research, termed neuroimmunology, which studies interactions of the nervous system and the immune system is emerging and two new journals (Brain, Behavior and Immunity and The Journal of Neuroimmunology) have been founded to report on specialized research in this area. For references, see the enclosed manuscripts. Based on recent studies performed during the tenure of this grant (see enclosed), the continued direction of these studies is the definition of the loci of action and the molecular mechanisms through which norepinephrine and associated molecules exert their effects on the immune system.

COMPLETED STUDIES

In recent years, Drs. Chambers and Perlman have formed a collaboration to study the effect of neuromodulators on immune function. This interaction grew out of Dr. Chambers' interest in immune regulation and cyclic nucleotides and Dr. Perlman's interest in the function of the medulla and the sympathetic nervous system. Initial experiments, performed utilizing a mouse lymphocyte serum-free culture system, allowing for precise definition of the extracellular environment, revealed that norepinephrine (NE) inhibited mitogen, antigen and cytokine stimulation of both T cells and B cells (see enclosed paper #1). Additional studies have suggested that although NE inhibits both T and B lymphocytes, the mechanisms through which NE-mediated inhibition occur probably differ in the two populations. Thus, cyclic AMP (cAMP) inhibits T cell proliferation, implying its use as a second messenger for NE action on the T cell, but the absence of a similar cAMP effect on B cells argues that NE-mediation of B cell proliferation could arise from cAMP-independent mechanisms. Pharmacological studies in our laboratories have shown that NE action on T cells is not inhibitable by α-adrenergic antagonists making it likely that NE transmits its effects through the β-adrenergic receptor, known to be present on both T and B cells. Of interest and unexpectedly, NE action on lymphocytes could not be blocked by the classic antagonist of the β-adrenergic receptor, propranolol. Nor could such action be inhibited by antagonists of the β_1, β_2 or β_3 species of the β-adrenergic family, perhaps implying the presence of a new receptor or altered receptor specific to lymphocytes.

In order to more specifically define the interaction of NE and cAMP in T cells, we made use of a mouse-derived T cell lymphosarcoma cell line, the S49 cell and S49 cell mutants unable to synthesize cAMP (AD CY^-) or unable to utilize the cAMP-dependent protein kinase (KIN A^-). NE inhibited wild type cells but did not affect the AD CY^- or KIN A^- mutants, suggesting that the NE-mediated inhibitory effect in T cells is modulated through the cAMP-protein kinase system. Propranolol, as with normal T cells, was unable

to antagonize the NE effects in S49 cells. In addition, we have utilized these same cells to show that NE and cAMP down-regulation of the Thy-1 gene (a gene specifying Thy-1 protein, a cell surface molecule expressed on lymphocytes and on brain cells) is also mediated through the cAMP-protein kinase A system; allowing us to study not only NE-associated cellular events but also NE-associated gene regulation. The use of these somatic cell mutants in this proposal will continue to provide us with powerful tools allowing dissection of some of the parameters of NE control, both at the cellular and gene level. Recent very preliminary studies in normal T cells suggests that NE may also down-regulate expression of the oncogene c-myc during the lymphocyte activation process, revealing the possible association of NF with the control of oncogenes.

During the course of our experiments, our mouse colony became infected with murine hepatitis virus (MHV) affording us the opportunity to study the effects of MHV on NE regulation of immune function (see paper #2). The ensuing investigations revealed that MHV infection led to diminution of mitogenic responses in spleen T lymphocytes but had no effect on either thymocytes or spleen B lymphocytes. MHV also totally ablated the NE regulatory loop in spleen T cells without affecting the other populations of lymphocytes. Interestingly, the viral effects on the immune function were evident much before detection of virus by serology. Knowledge of the specificity of the virus, both in terms of the affected cell and the particular NE regulatory loop, may prove important in understanding viral-neuroimmune interactions.

Most recently, utilizing a murine model system for anti-tumor immunity (MOPC 315 system) we have extended our studies of the relationships of NE to immune surveillance mechanisms in the control of anti-tumor immunity (see enclosed paper #3), an area of increasing importance to both the generation of neoplasia and its treatment. Here too, NE behaved similarly to our observations with spleen and thymic lymphocytes in that it inhibited the ability of immune cells to mount an anti-tumor response. In this case, also, propranolol did not block the NE effect.

In addition to the studies with the immune system, we also investigated effects of NE on keratinocytes in culture. In contrast to parallel studies with lymphocytes, addition of NE to cultures of keratinocytes appeared to enhance their proliferation. Recent studies suggesting the presence of nerve endings ending in the epidermis, taken in conjunction with these studies, suggest that neuromodulation may play additional roles in the regulation of epidermal homeostasis.

Finally, we expanded our studies of the effects of NE and associated molecules to include investigation at the level of the gene. Accordingly, we examined the effects of NE and cyclic AMP on the regulation of the Thy-1 gene and its gene product, Thy-1 protein. Thy-1 protein is a member of the immune super gene family and although its function eludes us, Williams (J. Theoret. Biol., *98*, 221, 1982) has suggested it plays a role in specific cell recognition. In our studies, addition of NE and/or cyclic AMP led to down regulation of Thy-1 protein in chromaffin cells (PC 12 cells), keratinocytes and lymphocytes (Chambers, et al., in preparation). When such studies were carried out in S49 T cell lymphosarcoma cells, only the wild type cell responded to cAMP, whereas the cyclic AMP-dependent protein

kinase mutant lost its ability to regulate Thy-1. Further studies, utilizing Northern blots, revealed that cAMP and presumably NE regulation of Thy-1 protein expression is at the level of gene transcription and most likely requires a trans-acting phosphorylated protein factor for negative control of gene expression (S. Lancaster and D.A. Chambers, in preparation, 1991; S. Lancaster, MS Thesis, Department of Biochemistry, University of Illinois at Chicago, 1991).

In summary, studies carried out during the tenure of this grant have revealed:

1) NE inhibits lymphocyte activation in the G1 period of both T cells and B cells and this inhibition is not antagonized by classical β-adrenergic receptor antagonists. NE modulation of lymphocyte activation may differ in T and B cells, and that T cell modulation occurs through a cAMP axis in contrast to B cell modulation.

2) NE inhibits the proliferation of normal S49 lymphosarcoma cells but not of either adenyl cyclase mutants or cAMP-dependent protein kinase mutants, supporting the presence of a phosphorylated protein regulation molecule. β-adrenergic antagonists could not reverse this effect.

3) NE inhibited the ability of lymphocytes to mount an anti-tumor response in the MOPC 315 syngeneic tumor model system and this inhibition was resistant to the addition of β-adrenergic antagonist.

4) In contrast to its effects on lymphocytes, NE stimulated the proliferation of neonatal mouse keratinocytes in culture.

5) Studies at the gene level revealed that NE probably modulates down-regulation of Thy-1 gene expression at the level of transcription through a phosphorylated protein regulation.

6) Infection of BALB/c mouse colonies with murine hepatitis virus (MHV) led to the marked inhibition of spleen T cell activation, but not spleen B cell activation a thymic T cell activation and the total ablation of the ability of NE to modulate the spleen T lymphocyte response.

In conclusion, the studies we have briefly outlined above, supply a foundation for further studies of the mechanisms of action that underlie the modulating effects of catecholamines on immune functions. They suggest experiments which will establish new insights into the inter-relationships between the immune system and the nervous system, potentially providing a molecular mechanistic framework for the molecular and cellular reactions which underlie the "stress" response and its relationship to pathology. Additionally, the knowledge gained from these studies could open new avenues for the design of molecular therapeutic approaches leading to rational therapies for stress-associated diseases.

ABSTRACTS

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J. Dent. Res. 66:172, 1987

Biological Response Modifier Effects on IL2-Mediated Lymphocyte Activation.

R.HAYDEN*, P.S.JACOBSON, R.L.PERLMAN and D.A.CHAMBERS.
University of Illinois at Chicago, Chicago, IL 60612

The regulation of immune cells in the periodontium has received increasing attention in recent years. Interleukin 2 (IL-2) is a lymphokine (secreted by T lymphocytes) which functions in the control of lymphocyte activation. These studies investigate the effects of biological response modifiers, previously related to lectin-mediated lymphocyte activation, on IL2-mediated lymphocyte activation in a serum and protein-free system. Previous studies in this laboratory revealed that cyclic AMP (cAMP), minoxidil and norepinephrine inhibit ConA-mediated lymphocyte activation. These experiments test the effects of these biological response modifiers on IL2-dependent lymphocyte activation. Mouse spleen cells were obtained as previously described (Cell 3 375, 1974) and cultured in microtiter plates in RPMI-1640 at 37°C in a humidified CO_2 atmosphere. Addition of 500 units of recombinant IL2 to $8x10^5$ cells resulted in a 60-100 fold increase in (^3H) thymidine incorporation into acid-precipitable DNA (eg. 1000 cpm vs. 100,000cpm), commencing 24 hours after IL2 addition. IL2-lymphocyte activation showed similiar time courses and stimulation indices as ConA-lymphocyte activation. Addition of $5x10^{-4}$M dibutyryl cAMP, 10ug minoxidil or $8x10^{-5}$M norepinephrine markedly inhibited IL2 activation (95%, 50% and 60% respectively) and showed similar effects in a ConA-activated system. These results show: 1) serum-free lymphocyte systems can be used to study the complex interactions between IL2 and biologic response modifiers and 2) suggest that the inhibitory effects of cAMP, minoxidil and norepinephrine occur at points distal to IL2 interaction in the lymphocyte activation cascade.

Fed. Proc. 46: 767, 1987

Biological Response Modifiers and IL2-Mediated Lymphocyte Activation. D.A. Chambers, R. Hayden, P.S. Jacobson, R.L. Perlman. University of Illinois at Chicago, Chicago, IL 60680.

Interleukin 2 (IL-2) is a lymphokine (secreted by T lymphocytes) which functions in the control of lymphocyte activation. These studies investigate the effects of biological response modifiers, previously related to lectin-mediated lymphocyte activation, on IL2-mediated lymphocyte activation in a serum and protein-free system. Previous studies in this laboratory revealed that cyclic AMP (cAMP), minoxidil and norepinephrine inhibit ConA-mediated lymphocyte activation. Balb/c mouse spleen cells were obtained as previously described (Cell 3 375, 1974) and cultured in microtiter plates in RPMI-1640 at 37°C in a humidified CO_2 atmosphere. Addition of 500 units of recombinant rat IL2 to $8x10^5$ cells resulted in a 60-100 fold increase in (^3H) thymidine incorporation into acid-precipitable DNA (eg. 1000 cpm vs. 100,000 cpm), commencing 24 hours after IL2 addition. IL2-lymphocyte activation showed similar time courses and stimulation indices as ConA-lymphocyte activation. Addition of $5x10^{-4}$M dibutyryl cAMP, 10μg minoxidil or $8x10^{-5}$M norepinephrine markedly inhibited IL2 activation (95%, 50% and 60% respectively) and showed similar effects in a ConA-activated system. These results show: 1) serum-free lymphocyte systems can be used to study the complex interactions between IL2 and biologic response modifiers and 2) suggest that the inhibitory effects of cAMP, minoxidil and norepinephrine occur at points distal to IL2 interaction in the lymphocyte activation cascade.

benzodiazepine (BZ) treatment. Altered GABA function may result from uncoupling of GABA and BZ binding sites, reflected in a change in GABA facilitation of BZ binding. Rats were chronically treated 4 wk with FZP in the drinking water. Regional brain dissections were made from chronically treated rats, matched controls or acutely pretreated rats (10 mg/kg diazepam; DZP) and stored at -70°C. The effect of 10^{-5} GABA or 10^{-4} bicuculline on 3-250 nM DZP inhibition of 1 nM [^{3}H] Ro15-1788 binding was evaluated in cortex (CTX) hippocampus (HIP) and striatum (STR) in a lysed, 1X washed P_2. There were regional differences in DZP IC_{50} (CTX, 53.4; HIP, 42.9; STR, 37.0). Neither acute nor chronic treatment affected DZP IC_{50}, or the GABA or bicuculline shift. Stimulation of 0.5 nM flunitrazepam binding by 10^{-7} to 10^{-4} GABA was measured in 2X frozen/thawed, 3X washed membranes from CTX, HIP, STR, midbrain (MBR) and cerebellum (CRB) of control and 4 wk treated rats. There were small regional differences in maximal stimulation by GABA in control brains. Chronic treatment had no effect in CTX (33.8 ± 5.8% vs 31.1 ± 4.9%) or HIP (35.1 ± 2.6% vs 35.7 ± 2.3%). A small increase in stimulation was seen in STR (36.8 ± 1.5% vs 41.7 ± 0.8%) and CRB (39.9% vs 43.8%). GABA stimulation in midbrain decreased (30.3 ± 3.2% vs 21.0 ± 1.6%).

Supported by NIDA grants DA04075 and DA02194.

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FASEB J. 2:A311, 1988.

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NOREPINEPHRINE MODULATION OF T AND B-CELL PROLIFERATION.

J. Cook-Mills, P Jacobson, R. Perlman* and D.A. Chambers.
Univ. of Illinois at Chicago, and Univ. of Chicago*, 60612.

Neurotransmitters have recently been implicated in the immune response. These experiments examined the in vitro effects of norepinephrine (NE) on T-cell and B-cell proliferation. BALB/c mouse spleen or thymus cells were cultured in serum-free RPMI 1640 for 48 hr. DNA synthesis was assayed by the incorporation of (^{3}H)thymidine for 4 hr into DNA. NE (10^{-5} M) inhibited splenocyte proliferation (50% of controls, 97,000 vs 200,000 cpm) or thymocyte proliferation (50% of controls, 32,000 vs 62,000 cpm) in response to the T-cell mitogen, ConA (1ug/ml). NE also inhibited LPS (a B-cell mitogen, 12ug/ml) stimulated proliferation in splenocytes (50% of controls, 14,000 vs 31,000 cpm) and 6-8 week old BALB/c nude mouse splenocytes (58% of controls, 5,000 vs 9,000 cpm). Inhibition of proliferation was observed when NE was added 0-20 hr after ConA or LPS addition. NE also inhibited (^{3}H)thymidine incorporation by nonstimulated splenocytes. Cell viability was 70-100% in all experiments. Others have related E responses to cAMP. Dibutryl-cAMP ($5x10^{-4}$M) inhibited (11% f controls) cell proliferation when added 0-8 hr after ConA addition, but did not inhibit LPS-mediated proliferation by cells obtained from nude or normal BALB/c mice. These results suggest that NE inhibition of lymphocyte activation occurs through a cAMP-mediated mechanism in T-lymphocytes and through other mechanisms in B-lymphocytes.

Supported by ONR N00179.

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VERAPAMIL BLOCKS SYN

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IMPAIRMENT OF LEARNING BEHAVIOR IN RATS BY NMDA RECEPTOR ANTAGONISTS: RADIAL MAZE AND PASSIVE AVOIDANCE TESTS. W

90

AMINO ACID MEDIATED

3156 Release of Human Platelet Surface Glycoprotein by Phosphatidylinositol Specific Phospholipase(s) C: A. Ohar and S.D. Shukla, Department of Pharmacology, University of Missouri, School of Medicine, Columbia, MO 65212.

We examined whether surface glycoprotein(s) of human platelets can be released by phosphatidylinositol specific phospholipase C (Plase C) treatments. Human blood platelets were isolated by low speed centrifugation and surface glycoproteins were labelled with ^{3}H-borohydride. Intact surface labelled platelets were treated with Plase C isolated from Staphylococcus aureus (SA) or Bacillus thuringiensis (BT). The released soluble components were separated by SDS-PAGE under reduced conditions and the protein patterns were obtained by fluorography. The regions of the gel corresponding to radioactive bands were cut out, dissolved in perchloric acid/H_2O_2 and counted. This study revealed that there was a significant release of a specific glycoprotein of M_r - 150 K in the medium due to Plase C treatment. In the course of this study, it was also observed that prolonged incubation of platelets in 0.25 M sucrose medium also caused release of this protein. We therefore depleted NaCl concentrations in the medium, gradually, with a concomitant increase in sucrose concentrations and observed that depletion of NaCl also affected the release of this glycoprotein in the medium. Thus sodium depletion in the medium perhaps causes activation of an endogenous Plase C which also cleaves the PI anchored to this glycoprotein. In conclusion, it is suggested that the M_r - 150 K glycoprotein is anchored in human platelet membrane via phosphatidylinositol. (Supported in part by NIH grant DK 35170)

3157 Release of Leukotriene D_4 Dipeptidase from Lung Membrane by Phosphatidylinositol-specific Phospholipase C. B. J. Campbell, S. F. Baker, S. D. Shukla, L. J. Forrester, and W. L. Zahler, University of Missouri, Columbia, Mo.

Frozen sheep lung tissue was homogenized in 0.33 M sucrose, 50 mM Hepes buffer at pH 7.5. The resulting homogenate was centrifuged at 8000 g for 15 min, and the supernatant obtained was further centrifuged at 26,000 g for 2 hr. The microsomal pellet was resuspended in 10 mM Hepes at pH 7.0 and centrifuged at 31,000 g for 1.5 hr. The membrane pellet was taken up in 10 mM Hepes buffer at pH 7.0 and treated with phosphatidylinositol-specific phospholipase C (PI-PLC) purified from Staphylococcus aureus. The release of leukotriene D_4 dipeptidase was followed by spectrophotometric procedures using glycyldehydrophenylalanine as the assay substrate. The activity of the released enzyme against leukotriene D_4 was determined by measuring the production of glycine from the sulfidopeptide leukotriene by pre-column derivatization of the amino acid with phenylisothiocyanate followed by high-performance liquid chromatography. When lung membrane was incubated with PI-PLC at a level of 1.5 units/ml at 37°, a gradual release of leukotriene D_4 dipeptidase occured reaching a level of 83% solubilized in 5 hrs. The release of the dipeptidase was shown to depend upon the amount of PI-PLC added to the incubation mixture with 85% dipeptidase released by 5 units PI-PLC/ml over 2 hrs. The specific activity of the solubilized enzyme was increased 48 fold from the lung homogenate by the PI-PLC digestion. The results indicate that leukotriene D_4 dipeptidase is anchored to the lung membranes by a covalent attachment to phosphatidylinositol.

J. Cell Biol. 107:559a, 1988.

3158 Why Do L Cells Fail to Express Glycophospholipid-Anchored Proteins to Their Surface? N. Singh, D. Singleton, and A. Tzagoloff, Case Western Reserve University School of Medicine, 2085 Adelbert Road, Cleveland, OH 44106.

Mouse L cells (LM-TK$^-$) secrete and do not express on their surface the normally glycophospholipid (GPL)-anchored protein, Ly6E.1. When genes or cDNAs encoding any of several other GPL-anchored membrane proteins (N-CAM 120, Qa, etc.) are transfected into L cells, stable transformants secrete the corresponding proteins and do not express them on their surface. To analyze the nature of this "anchoring defect," pair-wise fusions (with PEG) were performed between L cells and recessive mouse Thy-1$^-$ mutants of classes A-F (from R.Hyman) and BW1.2 (from K.Rock; BW1.2 complements classes A-F). Transient heterokaryons were examined for surface expression of Thy-1 and Ly6 after 24 hrs. by immunofluorescence. Hybrids of L cells and class A, B, C, E, F and BW1.2 express both surface antigens. Hybrids with class D (which does not synthesize Thy-1) express only Ly6. When the Thy-1$^-$ mutant, TlMl-C, was treated with the demethylating agent 5'-azacytidine, 10-15% of the cells became Thy-1$^+$ after 8 days. L cells failed to express Ly6 after similar treatment. We conclude that the anchoring defect in L cells is recessive and distinct from that of lymphoma mutants. The defect appears not to result from gene overmethylation.

-Supported by NIH grants.

3159 Modulation of Thy-1 Protein Expression in Murine Lymphocytes and Epidermal Cells. DA Chambers, RL Cohen, J Cook-Mills and PS Jacobson. Univ. of Illinois at Chicago.

Thy-1 is a cell surface glycoprotein (immunoglobulin superfamily) expressed in the mouse by T-cells, nerve, fibroblasts and epidermal cells (EC). The function of Thy-1 is unknown, but it is thought to play a role in intercellular communication. These experiments were designed to investigate modulation of Thy-1 gene expression in lymphocytes and EC by the regulatory molecules cAMP and norepinephrine (NE). Epidermal cells and spleen cells were harvested from BALB/c mice and incubated in RPMI 1640 in the presence and absence of cAMP (5×10^{-4}M, 10^{-3}M), cholera toxin (10^{-9}M) or NE (10^{-6}M, 10^{-5}M). EC were cultured up to 10 days in the presence of 13% FCS, and lymphocytes were cultured for 2 days in serum-free media. Cells were then incubated with FITC labeled anti-Thy-1.2 Ab and prepared for immunofluorescence microscopy or FACS. When compared to their untreated counterparts, treated EC and lymphocytes showed as much as 50% reduction in Thy-1 expression (EC: untreated 2-7% vs treated 1-3%; lymphocytes: untreated 40% vs treated 29%). In lymphocytes decreased Thy-1 expression correlated with inhibition of response to the mitogen ConA. These studies suggest that cAMP or NE modulation of Thy-1 gene expression may have significance in signal transduction in both the immune system and the skin. Supported by HHS grant AM33067.

3160 Mouse L^d Antigens in Glycosylation-defective CHO: A System for the Study of the Effect of Glycosylation on Expression and Lateral Diffusion of Membrane Glycoproteins. S. E. Barbour and M. Ediidin. The Johns Hopkins University, Baltimore, MD 21218.

The mouse L^d antigen is N-glycosylated on each of three external domains. Fluorescence Photobleaching Recovery (FPR) studies have shown that diffusion coefficients (D) for L^d mutants lacking one or more of their N-linked glycosylation sites are greater than for wild type L^d and vary inversely with the degree of glycosylation. These data indicate that carbohydrate moieties can constrain the lateral diffusion of L^d. This constraint could be mediated by the interactions of the L^d molecules with other glycosylated moieties on the cell surface. To approach this question, we have transfected wild-type and mutant L^d genes into Chinese hamster ovary cells (CHO) with various defects in N- or O-linked glycosylation. Cell surface expression of the L^d molecule was evaluated by flow cytometry. Wild type CHO expressed the wild type L^d molecule at levels comparable to those of the transfected L cell line 27.5.27. CHO mutants with reduced sialylation of complex carbohydrate or an overall reduction in complex carbohydrate expressed glycosylation-negative L^d molecules at higher levels than the wild type CHO. In all cases, expression of the wild type L^d was lower in the CHO mutants. Preliminary FPR data indicate that reduction of cell surface complex carbohydrate decreases the mobile fraction of the wild type L^d antigen in at least one mutant CHO cell line.

3161 Spontaneous and Lymphokine-Elicited Expression of Human Melanoma Cell-Associated Antigens by Cultured Epidermal Cells. LH Graf Jr, KA Kozlowski, V Mancino and JP Schredenti. Univ. of Illinois at Chicago.

Analyses using recombinant probes are facilitating molecular characterization of the distributions, modulation, structures and possible functions of tumor-associated antigens. Interspecific transfection and selective coamplification procedures have resulted in our isolation of recombinant clones for genes specifying 2 human melanoma-associated antigens (MAA) described by S. Ferrone and associates (N.Y. Medical College, Valhalla): "96K MAA" and "100K MAA." Each MAA is preferentially expressed by melanoma cells, has limited distribution on normal tissues and is modulated by lymphokines. Expression of 96K MAA and 100K MAA by human epidermal cells in culture was analyzed using a red cell immunorosetting assay to detect MAA$^+$ cells. Neonatal (foreskin) and adult epidermal melanocytes express 96K MAA at a frequency (f)$\leq$1% in the absence of inducers (i$^-$), at f$\geq$50% after 2-3 d exposure to immune interferon (IFN-γ), 200 units/ml, and at f$>$50% after 2-3 d treatment with mouse tumor necrosis factor-α, 20 ng/ml (neonatal melanocytes only). Neonatal and adult keratinocytes express 96K MAA at f$\leq$1% (i$^-$) and at f=5-10% (IFN-γ). Cultured melanocytes and keratinocytes express 100K MAA at f$\geq$50% (i$^-$), in contrast to the corresponding explanted adult cell populations, which are 100K MAA$^-$. Probes for 96K MAA and 100K MAA genes will aid in molecular study, respectively, of spontaneous and lymphokine-elicited expression of 96K MAA by normal and in vitro growth-transformed epidermal cells in culture, and of induction of 100K MAA expression by growth in culture. Supported by NIH grant CA44107.

Clin. Res. 37:411A, 1989.

REGULATION OF THY-1 PROTEIN EXPRESSION IN MURINE EPIDERMAL CELLS, LYMPHOCYTES AND PC12 CELLS. DA Chambers, RL Cohen, J Cook-Mills and PS Jacobson, Univ. of Illinois, Chicago, IL.

Thy-1 is a cell surface glycoprotein (immunoglobulin supergene family) expressed in the mouse by T-cells, nerve, fibroblasts and epidermal cells (EC). The function of Thy-1 is unknown, but it is thought to play a role in intercellular communication. These experiments were designed to investigate modulation of Thy-1 gene expression in lymphocytes, EC and PC12 cells by the regulatory molecules cAMP and norepinephrine (NE). Epidermal cells and spleen cells were harvested from BALB/c mice and incubated in RPMI 1640 in the presence and absence of cAMP (5×10^{-4}M, 10^{-3}M), cholera toxin (10^{-9}M) or NE (10^{-6}M, 10^{-5}M). EC and PC12 cells were cultured up to 10 days in the presence of FCS, and lymphocytes were cultured for 2 days in serum-free media. Cells were then incubated with FITC labeled anti-Thy-1.2 Ab and prepared for immunofluorescence microscopy or FACS. When compared to their untreated counterparts, treated EC, PC12 cells and lymphocytes showed as much as 50% reduction in Thy-1 expression (EC: untreated 2-7% vs. treated 1-3%; lymphocytes: untreated 40% vs treated 29%). PC12 cells cultured in the presence of either the 7S (2×10^{-10}M) or 2.5S (7.7×10^{-10}M) component of NGF express Thy-1. Treatment with cAMP, cholera toxin or NE results in a reduced, atypical pattern of Thy-1 expression compared with controls. In lymphocytes decreased Thy-1 expression correlated with inhibition of response to the mitogen ConA. These studies suggest that cAMP or NE modulation of Thy-1 gene expression may have significance in signal transduction in both the immune system and the skin.

FASEB J. 4: A2046, 1990.

2040 MURINE HEPATITIS VIRUS (MHV) INFECTION BLOCKS NOREPINEPHRINE (NE) INHIBITION OF T-CELL BUT NOT B-CELL PROLIFERATION. J. Cook-Mills, R. Perlman* and D.A. Chambers. Univ. of IL at Chicago, and Univ. of Chicago*, 60612.

NE is an immunomodulator in nervous-immune system communication. These experiments studied the in vitro effects of NE on proliferationof lymphocytes from BALB/c mice periodically infected by natural outbreaks of MHV at our institution. MHV is a common viral infection of conventionally housed mice. Murine serum anti-MHV antibodies were detected by ELISA. Spleen cells were cultured in serum-free RPMI-1640 for 48 hours followed by incorporation of ^{3}H-thymidine for 4 hours. Reagents used were the T-cell mitogen Concanavalin A (ConA, 0.5μg/ml), the B-cell mitogen lipopolysaccharide (LPS, 12μg/ml), NE, and the second messenger for NE signal transduction in T-cells dibutyryl(DB)-cAMP ($5x10^{-4}$M). For noninfected mice, NE (10^{-4}M and $5x10^{-4}$M) inhibited (90% and 50%, respectively) ConA- and LPS-stimulated spleen cell DNA synthesis. DB-cAMP inhibited (78%) ConA- but not LPS-stimulated DNA synthesis. In contrast, for MHV-infected mice, NE (10^{-4}M) inhibited (90%) LPS- but not ConA-stimulated spleen cell DNA synthesis. DBcAMP had no effect on ConA- or LPS-stimulated DNA synthesis for MHV-infected mice. Furthermore, ConA-stimulated DNA synthesis by spleen cells from MHV-infected mice was 50% that for noninfected mice. These studies suggest that the mechanism of MHV immunosuppression is primarily confined to T-cells and may act via the NE/cAMP axis. (Supported by ONR N00179)

2041 BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF THE MU-OPIOID RECEPTOR BINDING SITE ON CELLS OF THE IMMUNE SYSTEM. A.R.T. Radulescu,* B.R. DeCosta*, A.E. Jacobson,* K.C. Rice,* J.M. Blalock,* and D.J.J. Carr.* *Univ. Muenster Med. Sch., Muenster, FRG; *NIH, NIDDK, Bethesda, MD 20892; *Univ. Alabama at Birmingham, Birmingham, AL 35294.

A large body of evidence has accumulated with regard to the immunomodulatory role of opioids. Therefore, an investigation was conducted in order to characterize the functional and biochemical properties of the mu opioid receptor binding site on cells of the immune system. Using the mu-selective ligand, [^{3}H]-2-(p-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidazole (BIT), the specific in situ labeling of a protein with a Mr of 58 kD was observed on immune cells and brain tissue as determined by polyacrylamide gel electrophoresis and autoradiography. The binding is distributed evenly among B- and T-enriched populations. In addition, it has also been found on the macrophage cell line, P388d$_1$. Although two mu-class specific ligands, [D-Ala^2,N-Me-Phe^4,Gly^5-ol] enkephalin (DAGO) and [N-MePhe^3,D-Pro^4] morphiceptin (PLO17) were inactive in modulating NK cell activity (whereas β-endorphin enhances), both compounds significantly suppressed mitogen-induced antibody production as assessed by ELISA. Collectively, the data underscore the significant role opioids may have in immune homeostasis.

2042 EXPRESSION OF THE CHORIONIC GONADOTROPIN β CHAIN GENE BY ACTIVATED HUMAN LYMPHOCYTES. J. Dillon, B.M. Gebhardt, R.D. LeBoeuf, F.S. Galin, and J.R. Blalock. Dept. Physiology & Biophysics, Univ. Alabama at Birmingham, AL 35294.

The purpose of this study was: (1) to confirm that the CG gene was transcribed in activated lymphocytes and (2) to compare the lymphocyte-derived CG cDNA sequence to the placental CG nucleotide sequence. Human peripheral blood lymphocyte mRNA was used for selective first strand cDNA synthesis using an oligonucleotide primer complementary to the 3' end of placental CG β mRNA. The reaction products from first strand cDNA synthesis were used in a polymerase chain reaction (PCR) to amplify the CG β cDNA using synthetic oligonucleotide primers complementary to the 5' and 3' ends of the placental CG sequence. The PCR reaction products were subjected to a Southern analysis using a ^{32}P-labelled placental CG β probe. The results of this analysis showed that an appropriately sized DNA (700 bp) was present in the lymphocyte-derived PCR products and in a CG secreting cell line (JAR). DNA sequence analysis is currently in progress to determine the degree of homology between the lymphocyte and placental CG. The results of this study should establish that activated human lymphocytes express authentic CG. Production of CG by alloantigen-stimulated lymphocytes surrounding the blastocyst may have important effects in modulating the maternal immune response and in providing a biochemical signal for blastocyst nidation.

2043 FURTHER STUDIES ON IL-1 BETA INDUCED CENTRAL NERVOUS SYSTEM DYSFUNCTION. James A. Martinez, Luz Claudio, Sandy Smith and Celia F. Brosnan. Albert Einstein College of Medicine, Bronx, NY 10461.

Previous results from this lab have shown that intraocular injection of IL-1b in the rabbit results in a reversible delay in conduction that is associated with an acute inflammatory response. We have now pursued these studies further to determine the mechanisms involved in altered vascular permeability and it's effect on the ionic milieu of the retinal parenchyma. Quantitative ultrastructural studies demonstrate an increase in active transport, as defined by pinocytic vesicle profiles, that peaks at 3h PIC, decreases by 6h, and remains elevated at 24h PIC. At the height of the observed conduction deficits, large gaps are found between endothelial cells that are associated with hemorrhage and perivascular inflammation. Staining for altered cation binding, using the copper sulfate/potassium ferrocyanide technique, demonstrates increased reaction product associated with the epiretinal vessels and the inner plexiform layer. Redistribution of reaction product is also observed at some nodes of Ranvier adjacent to the internal limiting membrane. These observations support our conclusion that the IL-1b-induced conduction deficits in the rabbit retina are associated with the acute inflammatory response. Supported by USPHS grant # NS11920.

2044 THE DISTRIBUTION OF CALCITONIN GENE RELATED PEPTIDE IN THE THYMUS: AN IMMUNOCYTOCHEMICAL AND IN SITU HYBRIDIZATION STUDY. K. Bulloch, J. Hausman, T. Radoicic, S. Short, D.M. Simmons, L.W. Swanson*, Department of Psychiatry, UCSD, CA 92121, *Salk Institute, San Diego, CA 92138

Calcitonin gene related peptide (CGRP) is known to block Con A induced T cell proliferation. As a first step in determining the role of this peptide in T cell development and function we have studied the distribution of CGRP within mouse and rat thymuses and spleens utilizing immunochemistry and in situ hybridization. The results of this study show that CGRP is found in intrathymic nerves distributed to the corticomedullary boundaries adjacent to the vasculature with branches emanating into the cortical and medullary regions. Some fibers are invested in the arteries but the majority form varicosities among the cells of the thymus. CGRP is also found in a discrete population of cells located at the cortico-medullary boundary as well as in subcapsular and trabecular mast cells. In situ hybridization confirms that two populations of thymus cells synthesize CGRP messenger RNA in the thymus. Little to no CGRP was observed in the nerves or cells of the spleen. These findings confirm the biochemical analysis of CGRP carried out by Nilsson, 1989.

2045 THE EFFECTS OF HANDLING BALB/C MICE ON IMMUNOLOGICALLY RELEVANT PROCESSES. J. Moynihan, G. Brenner, N. Cohen, and R. Ader (SPON: N. Cohen). University of Rochester Medical Center, Rochester, NY 14642.

Picking up and holding female, group-housed BALB/c mice for two min and placing them in a holding cage for 0-4 min once/day for two weeks is associated with decreased responses to the T cell mitogen Concanavalin A and suppressed primary and secondary humoral responses to the T cell dependent antigen keyhole limpet hemocyanin (KLH). Handling does not effect the total number of spleen cells or splenic lymphocyte subpopulations. Handling results in significantly increased numbers of lung metastases following the iv injection of 10^5 syngeneic line 1 tumor cells (derived from a spontaneous alveolar carcinoma) that are sensitive to lysis by natural killer (NK) cells but do not elicit a strong T cell-mediated response. Surprisingly, handling does not alter splenic NK cell activity in vitro and in vivo assays of the clearance of ^{51}Cr-labelled line 1 cells from the lungs suggest enhanced NK activity in handled animals. Thus, the effect of handling on numbers of line 1 metastases is probably not due to decreased NK cell killing.

Interestingly, the numbers of metastases in handled and unhandled mice that were sympathectomized via injection of 6-hydroxydopamine (6-OHDA) did not differ from each other but were significantly elevated relative to vehicle-injected handled and unhandled animals. Further, 6-OHDA treatment, like handling, resulted in enhanced in vivo clearance of line 1 cells. These data suggest that the handling effect is not the result of a sympathetic nervous system response to stimuli associated with the handling manipulation.

1397

A SET OF HUMAN LYMPHOCYTE PUTATIVE G0/G1 SWITCH GENES INCLUDES GENES ENCODING HOMOLOGS OF RODENT ZINC FINGER PROTEINS AND A MURINE CYTOKINE.

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To generate cDNA libraries likely to be enriched in genes regulating the G0/G1 switch, quiescent G0 cells were cultured with a growth stimulant (e.g. lectin) and cycloheximide (Forsdyke,1985,Biochem.Biophys.Res.Comm.129,619; Lau & Nathans, 1985,EMBOJ 4,3145). The sets of genes identified by differential hybridization screening of rodent fibroblast libraries have included genes encoding zinc finger proteins, oncogenes, hormone receptors and cytokines (Rollins & Stiles,1989 ,1989,Adv.Canc.Res.53,1). The sets of genes identified by screening human lymphocyte libraries have been found to encode similar genes. One lymphocyte gene, G0S30, shows a simple pattern of bands by Southern blotting, indicating presence as a single copy in the human genome. The 501nt of 3' sequence (Genbank accession no: M24019) is homologous (87%) to zinc finger genes identified in rodent systems (e.g.Millbrandt, 1987, Science 238,797). These have been given various names (NGFIA, KROX24, ZIF/268, EGR-1). The mouse and rat genes show a similar degree of homology with each other. Thus, it appears that expression of the human equivalent of these genes is rapidly increased by lectin (or cycloheximide) in cultures of blood mononuclear cells. Run-on transcription studies indicate that the increase of G0S30 mRNA involves transcript stabilization. The increase of another mRNA (G0S19-1; M23452;Forsdyke,1985) is due, at least in part, to increased transcription. This gene, encoding a putative cytokine, is one of three genes in the human genome (M23178,M24110) which hybridize with G0S19-1 cDNA. Identical cDNAs have been identified in other human lymphocyte systems (LD78, Obaru et al.,1986,J.Biochem .99,885; AT464,Zipfel et al.,1989,J.Immunol. 142,1582).

1398

REGULATION OF THE γ CHAIN OF THE T-CELL ANTIGEN RECEPTOR

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T-cells express either of two antigen receptor types; one comprised of an α and β chain, the other a γ and δ chain. This exclusion occurs at least partly at the level of transcription; γ/δ cells express no α transcripts and only truncated β transcripts, similarly the major class of γ genes is not expressed in α/β cells (the δ gene lies within the α locus and is deleted). To elucidate the molecular events that control these TCR genes' rearrangement and expression, we have localized a strong, T-cell specific enhancer within a 450 bp fragment 3' of Cγ1. This enhancer sequence shared several similarities with the Cα enhancer and other previously described trans-activating factor binding motifs. CAT assays of transient transfectants have shown that this enhancer is not active solely in γδ-expressing T cell lines, but is very active in at least one α/β T-cell line. As the endogenous γ-gene is not normally expressed in αβ T-cells, other regulatory elements may exist. In line with this prediction, at least two distinct negative regulatory elements have been identified in sequences flanking the enhancer which nearly eliminate the enhancer activity in α/β but not γ/δ T-cells. These elements may participate in the commitment or maintenance of the differentiated T-cell phenotype. To explore this possibility, T cell subtype-specific nuclear factors that interact with the enhancer and negative regulatory elements are being characterized. (Supported by the NIH and an NSF Fellowship to DMS.)

1399 FASEB J. 4: A1934, 1990.

REGULATION OF THY-1 mRNA BY cAMP IN MURINE LYMPHOCYTES. S.A. Lancaster and D.A. Chambers. Center for Research in Periodontal Diseases and Oral Molecular Biology and Dept. of Biological Chemistry, University of Illinois, Chicago, IL 60612.

Thy-1 is a major glycoprotein expressed on the surfaces of murine T lymphocytes, most neurons, and fibroblasts. Expression of Thy-1 is regulated during differentiation, and Thy-1 might be involved in T cell proliferation and cell-cell recognition. Treatment of thymocytes with cAMP ($5x10^{-4}$M) decreases expression of Thy-1 cell-surface protein. The effects of dibutyryl cAMP ($5x10^{-4}$M) on Thy-1 mRNA levels in BALB/c murine thymocytes and in S49 cells (a BALB/c-derived lymphoma line) were investigated. RNA was extracted with guanidinium thiocyanate, isolated by sedimentation through CsCl, and purified by ethanol precipitations. Northern analysis was performed on the samples. Blots were hybridized with a ^{32}P-labeled Pst I fragment of a BALB/c genomic Thy-1.2 clone. Treatment of murine thymocytes or S49 cells with dibutyryl cAMP led to a decrease in cellular Thy-1 mRNA levels with no significant effect on levels of total RNA. In S49 cells, cellular Thy-1 mRNA levels decreased significantly after treatment for only 1-2 hours with dibutyryl cAMP. These results suggest that regulation of Thy-1 mRNA may involve cAMP. (Supported by ONR N00179.)

1400

CLONING OF A LYMPHOID-SPECIFIC NUCLEAR FACTOR THAT BINDS A MURINE RETROVIRAL PROMOTER . C. Gunther, J. Nye, R. Bryner, B. Graves (SPON: R. Schackmann). Univ. of Utah, Salt Lake City, UT 84132.

Our objective is to characterize lymphoid transcription factors by identifying proteins that regulate transcription of leukemic murine retroviruses. Transcription control elements of Moloney MSV LTR that function in T-lymphocytes have been mapped by deletion mutagenesis and transient expression assays in EL4 cells. Calf thymus nuclear extracts were screened for DNA binding activities specific to the MSV promoter element. We have identified one binding activity, termed lymphoid nuclear factor 1 (LNF1), whose DNaseI footprint spans a 20-30 bp region of the MSV LTR promoter. Methylation and ethylation interference data mapped LNF1's binding site to specific LTR nucleotide pairs. Base substitution at these most critical positions generated a mutant LTR promoter that lacks the ability to bind LNF1. In transient expression assays in EL4 cells, this mutant promoter transcribes a reporter gene 20X less efficiently than a wild-type promoter. We conclude that the LNF1 binding site is a positive control element of the MSV LTR. The LNF1 binding site has been used as a sequence-specific DNA probe for screening a mouse thymus cDNA expression library. One cDNA clone that binds the probe has been isolated. Southwestern blots have identified a 30kD polypeptide encoded by the cDNA that binds an LNF1 binding site and does not bind a mutant LNF1 site. In Northern blot analysis, high levels of mRNA with homology to the cDNA sequences were detected only in lymphoid tissues. Deletion mutagenesis of the thymus cDNA clone is underway in an effort to map the DNA binding domain of the encoded polypeptide. [Supported by NIH-CA09602, MOD#5-676, NIH-GM38663.]

1401

THE PROMOTER STRUCTURE OF THE HUMAN ICAM-2 GENE.

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We have analysed the promoter structure of the gene for human intercellular adhesion molecule 2 (ICAM-2). Using primer extension analysis of cytoplasmic RNA from the human T cell leukemia line, Jurkat, we have identified the start site for mRNA transcription at approximately 105bp upstream of the translational srtart. Thirty base pairs 5' of the putative transcription start is an atypical TATA box. Another sixty base pairs 5' of the TATA box is a potential binding site for the general transcription factor, Sp1. We are presently doing DNAse I footprint analysis to confirm these sites are bound by the appropriate transcription factors as well as identify other sites for DNA binding proteins.

The primer extension analysis of cytoplasmic RNA also shows that Jurkat constitutively expresses ICAM-2 mRNA whereas a second T cell leukemia line, CEM, is negative for ICAM-2mRNA. These results were confirmed by northern blotting of the same RNA preparations. Upon induction of these cell lines to IL-2 secretion by PHA/PMA, ICAM-2 mRNA can now be detected in CEM. We are analyzing this further by preparing nuclear extracts from both cell lines before and after induction to test whether these preparations will have different footprints on the promoter under these different conditions and whether the activity of these extracts in *in vitro* transcription assays is altered.

1402

HORMONAL INFLUENCE ON CELL SURFACE ANTIGEN EXPRESSION OF MURINE SPLEEN CELLS. H. Rataiczak, R. Lance, P. Thomas, K. Hagen, J. Wu and P. Halberg. IIT Res. Inst., Chicago, IL 60616, U IL, Chicago, IL 60612 and U MN, Minneapolis, MN 55455.

The circadian rhythm of splenic cell surface antigens and serum levels of corticosterone and estradiol were studied in female C3HeB/FeJ mice. Mice were placed in 1 of 6 boxes with lights on 12/24 hr and onset of light in each box staggered by 4 hr. After acclimatization for 21 d, mice were anesthetized with CO_2, exsanguinated, and spleens removed asceptically. Hormones were evaluated in serum by radioimmune assay. Monoclonal antisera conjugated to fluorescein isothiocyanate were used to determine Thy 1.2, Lyt-2, L3T4 and immunoglobulin, defined by flow cytometry with an Epics V fluorescent activated cell sorter. Significant circadian rhythms were found for each cell surface antigen and for corticosterone. Correlations were significant for corticosterone levels and B cells and estradiol. In a separate experiment, mice were depleted of adrenals or ovaries and corticosterone or estradiol injected, respectively. Adrenalectomy or ovariectomy resulted in decreased % Thy 1.2-bearing cells and decreased numbers of B cells. When adrenalectomized mice were reconstituted with corticosterone, % Thy 1.2-bearing cells and absolute numbers of B cells were returned to normal.

1558

THE REDISTRIBUTION OF TUMOR INFILTRATING LYMPHOCYTES IN MICE FOLLOWING ADOPTIVE IMMUNOTHERAPY

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In murine models and in human patients with metastatic melanoma, tumor infiltrating lymphocytes (TIL) have been demonstrated to mediate regression of tumors. An understanding of TIL trafficking, particularly to sites of tumor deposits, may aid in elucidating the mechanism of the anti-tumor activity. These studies have examined two membrane-labelling compounds, PKH26 and PKH95, for their suitability as TIL tracking agents. PKH26 and PKH95 are very similar compounds, containing an aliphatic portion coupled to a red fluorochrome, but PKH95 contains an iodine atom that can be exchanged with radioactive isotopes for tracking or imaging. PKH26 is suitable for fluorescent tracking only. *In vitro* studies of murine TIL stained with up to 20 μM PKH26 or 5 μM PKH95 have not shown significant differences in proliferation, specificity or cytotoxic effector function associated with labelling. Adoptive transfer of murine TIL stained with either 20 μM PKH26 or 5 μM PKH95 were able to mediate regression of 3 to 5-day-old pulmonary metastases at similar cell numbers as unstained TIL. After establishing that PKH26 and PKH95 do not adversely effect TIL function, we used these compounds in TIL trafficking experiments. We have been able to detect PKH26 stained cells in lung and liver, by flow microfluorimetry, up to 6 days after adoptive transfer and have been able to detect ^{125}I-PKH95 labelled TIL in several organs at 7 days post injection. Other experiments examining effects of modulation of trafficking on TIL efficacy are in progress.

1559

GENERATION OF THERAPEUTIC T CELLS FROM TUMOR-BEARING MICE BY IN VITRO SENSITIZATION;FEASIBILITY OF CRYOPRESERVATION OF PRECURSOR AND EFFECTOR CELLS AND LONG-TERM CULTURE OF EFFECTOR CELLS. T.Chou, M.Nakashoshi, H.Miyao, A.Mizusawa, M.Mitsuma, H.Moriyama, Y.Itoh, and M.Ando. Dept.Med.(II),Niigata University School of Medicine, Niigata, Japan.

Using a weakly immunogenic murine fibrosarcoma, MCA 205, we have demonstrated that therapeutic cells can be generated by the in vitro sensitization culture where lymphocytes from tumor-bearing mice were cultured with viable tumor stimulator cells and low-dose recominant IL-2. In order to apply this adoptive immunotherapeutic approach to the clinic, we investigated the feasibility of cryopreservation of precursor and effector cells and long-term culture of the IVS cells. Tumor-bearing mice were created by the intrafootpad injection of $5x10^5$ tumor cells and 5-8 days after tumor injection, popliteal lymph nodes and spleen were harvested and cryopreserved. Tumor cells were also harvested from mice and cryopreserved. These cells were thawed and Responder lymphocytes($4x10^6$/well) were co-cultured with tumor stimulator cells ($2x10^6$/well) in the presence of 20U/ml RIL-2(TGP-5;kindly supplied by TAKEDA Pharm.). Tumor cell line was established and tested in the IVS culture to see if they can serve as antigenic stimulators. In vivo anti-tumor effect was evaluated by the adoptive immunotherapy of 3-day pulmonary metastases model. The IVS effector cells generated from frozen lymphocytes and tumor cells were effective in reducing No. of metastases. The IVS effector cells could be frozen without affecting their in vivo anti-tumor acitvity. Furthermore, the effector cells could be expanded by periodic antigenic stimulation and low-dose RIL-2 for more than 3 months. Our results demonstrated that adoptive immunotherapy protocol could be easily planned in combination with other therapies, such as chemotherapy and radiotherapy.

FASEB J. 5: A639, 1991

1560

EFFECT OF SYNTHETIC ALKYL-LYSO-PHOSPHATIDYLCHOLINE (ALP) ON IL-1 AND TNF PRODUCTIONS.

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The in vitro modulation of mouse and human monocyte-macrophages by ALP was investigated. When the P 388D1 cell line was pretreated with 10 μg/ml of various ALP, 5 FU, CIS or ARA-C for 24 h, washed and then incubated with 1 μg/ml LPS, the release of IL-1 was increased (Table).

Agonists	% increase in IL-1 production	
BN 52205	121 %	***
BN 52207	172 %	***
BN 52210	33 %	
BN 52211	76 %	*
BN 52215	31 %	
METHOXY PAF	76 %	*
5 FU	1170 %	***
CIS	377 %	***
ARA-C	431 %	***

* $p < 0.05$; *** $p < 0.001$

As well, the TNF activity released from LPS-stimulated human monocytes was increased in a bell-shape fashion with a maximal effect (4,592 %, 2,895 %) when the cells were incubated for 24 h in the presence of 2.5 μg/ml BN 52211 or BN 52205, respectively. The present results confirm that besides direct antitumoral effects, these ALP exhibit immunomodulatory activities.

1561

CATECHOLAMINE SUPPRESSION OF THE IN VITRO GENERATION OF ANTI-MOPC-315 SYNGENEIC PLASMACYTOMA CYTOTOXICITY. Joan M. Cook-Mills, Margalit Mokyr, Robert L. Perlman*, and Donald A. Chambers, Univ. of Illinois at Chicago, and Univ. of Chicago*.

Several immune responses are suppressed by stress-related molecules. The effects of these molecules on the immune response to syngeneic tumor cells was the focus of this study. BALB/c mouse spleen cells were cultured with stress-related molecules and mitomycin-C-treated MOPC-315 syngeneic plasmacytoma tumor cells for 3-5 days followed by assessment of anti-MOPC-315 cytotoxic activity. The generation of this cytotoxic activity was inhibited (50-90%) when norepinephrine (NE), isoproterenol (ISO) or epinephrine (10-100μM) was added 0-2 days after culture initiation. Dopamine and catechol but not serotonin or carbachol were also inhibitory. This inhibition was mimicked by DBcAMP, a second messenger analog, when added 0-4 days after culture initiation. Thus, catacholamines may suppress the generation of cytotoxicity against syngeneic tumor cells via a cAMP mediated mechanism. This suppression suggests that stress-related molecules may participate in regulation of anti-tumor defense mechanisms. (Supported by ONR N00179)

1562

ANTIPROLIFERATIVE EFFECT OF INTERLEUKIN-1 ON HUMAN OVARIAN CARCINOMA CELL LINE (NIH:OVCAR-3) P. L. Kilian, K. L. Kaffka, D. A. Biordi, J. A. Lioman, V. R. Benjamin, D. Feldman, C. A. Dinarello (SPON:M. K. Gately). Hoffmann-LaRoche, Nutley, NJ 07110

The human ovarian carcinoma cell line, NIH:OVCAR-3, possesses high affinity receptors for interleukin-1 (IL-1). Binding experiments with (^{125}I)IL-1 alpha indicate a dissociation constant of approximately 55 pM and the presence of approximately 7,800 receptors/cell. These receptors bind both IL-1 alpha and IL-1 beta and internalize IL-1. Half-maximal inhibition of NIH:OVCAR-3 cell proliferation is observed with 2-3 U/ml of IL-1 alpha or IL-1 beta. A maximal effect (80% inhibition of cell proliferation) is achieved by treatment of cells with ≥10 U/ml of IL-1 for 3 days. The antiproliferative effect of IL-1 is blocked by IL-1 receptor antagonist (IL-1ra). Light and electron microscopy studies show that IL-1 treatment causes cytopathologic changes in cells and a reduction in the number of mitotic figures in NIH:OVCAR-3. IL-1 stimulates PGE_2 release by NIH:OVCAR-3 cells, but this response is unrelated to the antiproliferative effect of IL-1. Interferon-alpha A (IFN-alpha A) also inhibits growth of NIH:OVCAR-3 cells in a concentration-dependent manner. Combination of IFN-alpha A and IL-1 gives a synergistic inhibition of NIH:OVCAR-3 cell proliferation. IL-1 alone or in combination with IFN-alpha A or other agents may be useful for treatment of human ovarian cancer.

FULL LENGTH PAPERS

Cook-Mills, J.M., Munshi, H.G., Perlman, R.L. and Chambers, D.A. (1991) Mouse Hepatitis Virus Infection Suppresses Modulation of Mouse Spleen T-Cell Activation, Immunology, accepted with revision.

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